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TAXONOMIC DESCRIPTION

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Thermococcus camini sp. nov., a hyperthermophilic and piezophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Atlantic Ridge

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Abstract

A coccoid-shaped, strictly anaerobic, hyperthermophilic and piezophilic organoheterotrophic archaeon, strain Iri35c^T, was isolated from a hydrothermal chimney rock sample collected at a depth of 2300 m at the Mid-Atlantic Ridge (Rainbow vent field). Cells of strain Iri35c^T grew at NaCl concentrations ranging from 1–5% (w/v) (optimum 2.0%), from pH 5.0 to 9.0 (optimum 7.0–7.5), at temperatures between 50 and 90 °C (optimum 75–80 °C) and at pressures from 0.1 to at least 50 MPa (optimum: 10–30 MPa). The novel isolate grew on complex organic substrates, such as yeast extract, tryptone, peptone or beef extract, preferentially in the presence of elemental sulphur or L-cystine; however, these molecules were not necessary for growth. Its genomic DNA G+C content was 54.63 mol%. The genome has been annotated and the metabolic predictions are in accordance with the metabolic characteristics of the strain and of *Thermococcales* in general. Phylogenetic analyses based on 16S rRNA gene sequences and concatenated ribosomal protein sequences showed that strain Iri35c^T belongs to the genus *Thermococcus,* and is closer to the species *T. celericrescens* and *T. siculi*. Average nucleotide identity scores and *in silico* DNA–DNA hybridization values between the genome of strain Iri35c^T and the genomes of the type species of the genus *Thermococcus* were below the species delineation threshold. Therefore, and considering the phenotypic data presented, strain Iri35c^T is suggested to represent a novel species, for which the name *Thermococcus camini* sp. nov. is proposed, with the type strain Iri35c^T (=UBOCC M-2026^T=DSM 111003^T).

Contrary to many orders within the *Archaea*, the order *Thermococcales* has led to the isolation of numerous cultured representatives that have been the subject of physiological and genomic studies. *Thermococcales* populate a variety of high-temperature natural ecosystems (deep-sea and shallow-marine hydrothermal vents, terrestrial hot springs, oil reservoirs, solfataric systems, etc.), the most important of which are marine hydrothermal vents, from which the largest number of isolates originate [1]. Among the three genera (*Thermococcus*, *Pyrococcus* and *Palaeococcus*) of the order *Thermococcales* [2], it is the genus *Thermococcus* that has led to the isolation of the largest number of species, with 33 species (with names validly recognized by the ICSP, the International Committee on Systematics of Prokaryotes) recorded to date. Physiological studies of these species have shown that this genus is composed of hyperthermophilic anaerobic taxa developing mainly through chemoorganoheterotrophy by coupling the oxidation of peptides or sugars to the reduction of elemental sulphur and protons [2]. This genus is also described to contain carboxydotrophic species (i.e. [3, 4]. Thanks to

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Abbreviations: ANI, average nucleotide identity; CAPSO, N-cyclohexyl-3-aminopropanesulfonic acid; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOMOPIPES, Homopiperazine-1,4-bis(2-ethanesulfonic acid); ICSP, International Committee on Systematics of Prokaryotes; MAR, mid-atlantic ridge; MES, 2-(N-morpholino)ethanesulfonic acid; PIPES, piperazine-N,N'-bis(2-ethanesulfonic acid); TAPS, N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid, [(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid; UBOCC, University of Brest (UBO) Culture Collection.
The DDBJ[/GenBank/ENA] accession number for the genome sequence of *Thermococcus camini* Iri35c^T sp. nov. is LR881183.1. The complete 16S rRNA gene sequence of *T. camini* sp. nov. strain Iri35c^T is available at GenBank/EMBL/DDBJ/PIR under accession no. MT921160.
One supplementary table and two supplementary figures are available with the online version of this article.

the different genetic tools available to work on models of *Thermococcus* species, such as *Thermococcus kodakarensis* or *Thermococcus barophilus* for example, many functional studies have been carried out and have allowed significant advances in our knowledge of their metabolism, genomic maintenance mechanisms and biological adaptations, instrumental in advancing our understanding of the biology of the *Thermococcales* and of the *Archaea* in general (*e.g.* [5, 6]. In addition to being important and ubiquitous players in the hot areas of the hydrothermal ecosystems, *Thermococcus* species are also of particular interest for learning more about the cellular processes at the limits of life, as this genus contains extremophilic and polyextremophilic organisms, adapted to one or more extreme physical or chemical conditions of their natural habitat. In this way, all taxa of the genus *Thermococcus* are hyperthermophilic, capable for some of them to divide up to a maximal temperature of 100 °C (*e.g. T. eurythermalis*, *T. kodakarensis*, *T. peptonophilus*) [7–9], tolerating in addition high doses of gamma radiation for some of them (*e.g. T. gammatolerans* resist up to 30 kGy of γ-radiation) [10], and with better growth under high hydrostatic pressure (*e.g. T. barophilus*, *T. piezophilus; T. piezophilus* holds the current record of pressure range for growth, growing from atmospheric pressure to 130 MPa) [11, 12] or under alkaline pH conditions for others (*e.g. T. alcaliphilus*) [13].

In this article, we describe a novel hyperthermophilic organoheterotrophic sulphur-reducer, strain Iri35c^T, isolated from a hydrothermal rock sample from the Rainbow vent field, at the Mid-Atlantic Ridge. Genotypic and phenotypic characteristics meet the standard nomenclatural criteria to delineate a novel species. We propose to name this new species *Thermoccocus camini*.

A chimney rock sample was collected at a depth of 2300 m from a hydrothermal vent at the Rainbow vent field (36°13′N, 33°54′W), at the Mid-Atlantic Ridge, in June 2001, during the IRIS oceanographic cruise. Onboard, the sample was preserved in a sealed sterile anoxic vial and stored at 4 °C, since the objective was to cultivate (hyper)thermophiles from this sample. Once in the lab, enrichment cultures followed by three series of dilutions-to-extinction were performed at 85 °C, in reduced TRM medium (pH 6.8), containing 5 g l^{-1} elemental sulphur, as described elsewhere [14]. A collection of pure strains was then deposited in the UBOCC collection (https://ent.univ-brest.fr/lm2e/home/#/), at −80 °C with 5% (v/v) DMSO. The strain Iri35c^T described here bears the accession number UBOCC-M-2026^T. The purity of this isolate was confirmed routinely by microscopic examination, and by sequencing of its genome.

The genomic DNA of Iri35c^T was extracted using a phenol-chloroform procedure. The TruSeq DNA PCR-free kit (Illumina, USA) was then used to prepare paired-end sequencing libraries with an average insert size of 550 nt, before the genome was sequenced using the Illumina's MiSeq technology (2×300 bp paired-reads, V3 chemistry), at the Marine Biological Laboratory (Woods Hole, MA, USA). Paired-end reads were filtered with the Python package illumina-utils using the command 'iu-filter-quality-minoche' and default parameters [15, 16]. The *de novo* assembly of the genome was carried out using CLC Genomics Workbench v8.5.1 (https://www.qiagenbioinformatics.com/products/clc-genomics-workbench). A total of 482309 read pairs of 300 bp were used for the genome assembly, representing a mean coverage of about 120×. The assembled genome was analysed and annotated with the MicroScope Microbial Genome Annotation and Analysis Platform (MaGe) (https://mage.genoscope.cns.fr/microscope/home/index.php) using KEGG and BioCyc databases [17]. It consists of one circular chromosome of 2022529 base pairs in size, and has a G+C content of 54.63 mol%. CheckM estimated the genome to be 100% complete based on the presence of default single-copy marker genes (four markers were missing) and without any contamination. The genome consists of 2204 encoding protein sequences, 46 tRNA genes, a single 16S-23S rRNA operon, two 5S rRNA, and 14 miscellaneous RNA genes. This genome is available in DDBJ/ENA/GenBank under the accession number LR881183.1 (BioProject: PRJEB40155).

Pairwise 16S rRNA gene sequence similarity was determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/ [18]). The 16S rRNA gene sequences were aligned using MAFFT v7.427 (parameters -maxiterate 1000 -localpair) [19], and the alignment was trimmed with BMGE v1.12 (default parameters) [20]. Then, PhyML v. 3.3.20190909 was used to build the tree thanks to the web-server http://www.atgc-montpellier.fr/phyml/ [21]. The evolutionary model was selected with the SMS algorithm [22] and the branch support was computed with the aLRT SH-like method. The tree was visualized with iToL [23] and rooted between the *Thermococcus* and *Pyrococcus* genera. Since the 16S rRNA gene sequence is highly conserved and therefore not very discriminating between *Thermoccocales*, a phylogenetic tree based on ribosomal proteins was also constructed. This phylogenomic tree was based on the concatenation of 49 ribosomal proteins shared by all genomes. Each protein was aligned and trimmed separately with MAFFT (parameters -globalpair -maxiterate 1000) and BMGE (default parameters), respectively. Then, each alignment block was concatenated into a single alignment that was submitted to PhyML. The evolutionary model was selected with SMS and the branch support was computed with the aLTR SH-like method. The tree was visualized with iToL and rooted between the *Thermococcus* and *Pyrococcus* branches. Average Nucleotide Identity scores (ANI) were calculated using two methods: OrthoANIu from the EzBioCloud web server (https://www.ezbiocloud.net/tools/ani) [24]; and ANIb values by JSpeciesWS Online Service ([25]; http://jspecies.ribohost.com/jspeciesws/#analyse). These ANI scores were calculated between the genome of strain Iri35c^T and the genomes of the four closest type species whose genomes are available: *T. celericrescens* TS2^T (NZ_LLYW00000000.1), *T. siculi* RG20^T (NZ_CP015103.1), *T. cleftensis* CL1^T (NC_018015.1) and *T. pacificus* P-4^T (NZ_CP015102.1). Digital DNA–DNA hybridization (dDDH) scores were also determined by the genome-to-genome distance calculator (GGDC 2.1), using the formula 2 [26].

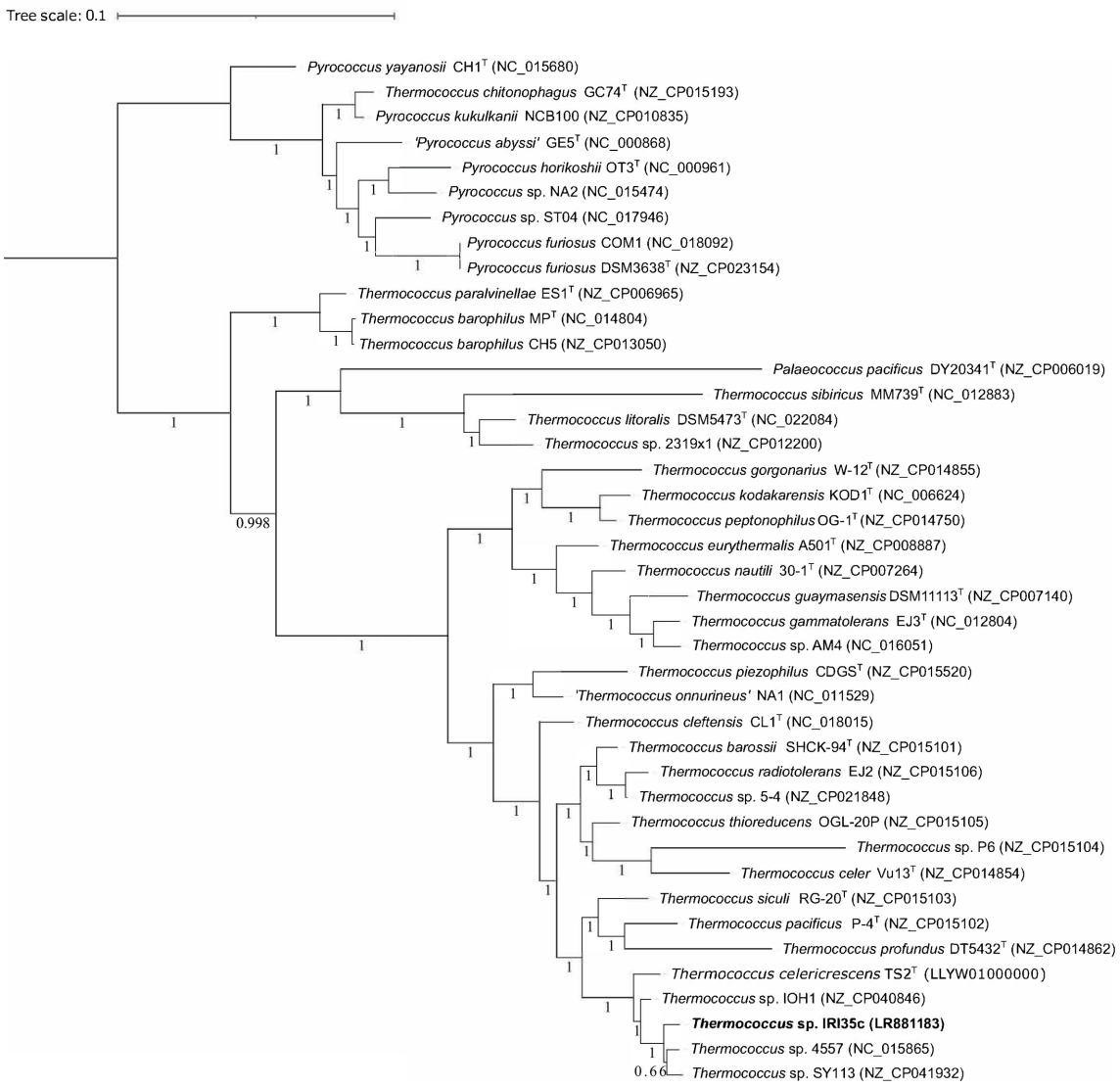

Fig. 1. Phylogenetic tree of the strain Iri35c^T and representatives of *Thermococcales*, based on 49 ribosomal proteins (Proteins associated with the large ribosomal subunit: L1, L2, L3, L4, L6, L7AE, L11, P1 (=L12P), L13, L15, L15E, L18, L18A, L18E, L19E, L21E, L22, L24, L29, L30, L30E, L31E, L32E, L37AE, L37E, L39E, L40E, L44E; Proteins associated with the small ribosomal subunit: S2, S3, S3AE, S4, S4E, S5, S6E, S7, S8, S8E, S9, S10, S12, S13, S15, S17, S17E, S19, S19E, S27E, S28E). The tree was built by maximum-likelihood (PhyML). Branch support, shown on the tree, was computed with the aLTR SH-like method. Bar, 0.1 amino-acid substitution rate.

Phylogenetic analyses of the 16S rRNA gene sequences and concatenated ribosomal proteins both confirmed that the new isolate branched within the archaeal genus *Thermococcus* (Figs 1 and S1, available in the online version of this article). The most closely related species of strain Iri35c^T were *Thermococcus celericrescens* TS2^T (99.66% 16S rRNA gene sequence similarity), *T. siculi* RG-20^T (99.26%), *T. barossii* SHCK-94^T (98.79%), *T. thioreducens* DSM 14981^T (98.79%), *T. hydrothermalis* AL662^T (98.75%), *T. cleftensis* CL1^T (98.59%) and *T. pacificus* P-4^T (98.05%). Due to the high degree of similarity between the sequences of 16S rRNA genes, overall genomes relatedness indices were calculated. The genomes of strain Iri35c^T and of its closest relatives shared OrthoANIu values ranging from 79.45–88.20% (Tables 1 and S1), and ANIb values between 78.68 and 87.31% (Table 1). These values are far below the ANI value of 95–96% generally accepted as a boundary for species delineation [27]. Digital DNA–DNA hybridization scores were also well below the DDH threshold level for species demarcation (70%), with values from 22.20–35.10% between the genome of strain Iri35c^T and the genomes of its closest neighbours, respectively (Tables 1 and S1) [28]. These results based on standard genomes relatedness indexes provide evidence that strain Iri35c^T represents a new genomic species [29].

Morphological characteristics of strain Iri35c^T were determined by light microscopy (Olympus BX60 and CX40) and scanning electron microscopy (FEI Quanta 200) (Fig. S2).

Table 1. Characteristics differentiating strain Iri35c^T from closest species of the genus *Thermococcus*

Strains: 1, Iri35c^T (data from this study); 2, *T. celericrescens* TS2^T [38]; 3, *T. siculi* RG20^T [39] ; 4, *T. cleftensis* CL1^T [40, 41]; 5, *T. pacificus* P-4^T [32]. Characteristics are scored as: +, positive ; –, negative; S, stimulatory; R, required; ND, not determined.

Characteristics	1	2	3	4	5
Geographical origin	Hydrothermal chimney, Mid-Atlantic Ridge	Hydrothermal chimney, Suiyo Seamount	Hydrothermal fluid, Mid-Okinawa Trough	Hydrothermal worm, Juan de Fuca Ridge	Geothermally heated bottom deposits in a bay in New-Zealand
Depth (m)	2300	1380	1394	2350	40
Growth optimum:*					
Temperature (°C)	75–80	80	85	88	80–88
pH	7.0–7.5	7.0	7.0	ND	6.5
NaCl (%)	2	3	2	ND	2.0–3.5
Doubling time (min)*	80	20	130	42	ND
Sulphur requirement†	S	S	S	S	R
Growth on:†					
Maltose	+ (weak)	–	–	+	–
DNA G+C content (mol%)*	54.63	54.6	55.8	55.8	53.3
16S rRNA gene sequence similarity	100	99.66	99.26	98.59	98.05
OrthoANIu (%)	100	88.20	79.72	82.50	79.45
ANIb (%)	100	87.31	78.93	81.51	78.68
dDDH (%)	100	35.10	22.40	24.90	22.20

*Data from the literature.
†Data obtained for the five strains under same experimental laboratory conditions.

Cells were motile cocci that occurred generally singly and divided by constriction (Fig. S2). Under optimal growth conditions and in the mid-exponential phase of growth, cells occurred as irregular cocci of 0.8–1.7 µm in diameter (mean 1.1±0.2 µm, *n*=31).

Unless stated otherwise, physiological assays were carried out anaerobically (N_2 headspace) in modified Ravot medium, at 80 °C, in duplicates, in the presence of elemental sulphur, as described elsewhere [11]. Growth tests were generally carried out as described previously [11]. Cells were routinely counted by direct cell counting using a modified Thoma chamber (depth 10 µm), and checked by flow cytometry (CyFlowSpace, Sysmex Partec, GmbH, Görlitz, Germany). Cells were fixed with 2.5% (v/v) glutaraldehyde (Sigma) and stored at −80 °C, before counting by the two methods described above. Determination of the temperature range for growth was carried out at 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 °C. The isolate was hyperthermophilic and grew between 50 and 90 °C with an optimum at 75–80 °C. Salt tolerance was tested at 80 °C with various concentrations of NaCl (0, 1, 2, 3, 4, 5, 6, 7 and 8%, w/v). Strain Iri35c^T required NaCl for growth and grew at NaCl concentrations between 1 and 5% (optimum: 2%). The pH range for growth was tested from pH 3.0 to pH 10.0 (initial pH at 20 °C) with increments to one unit near the limits of the pH range, and with increments of 0.5 unit around the optimum. For this experiment, we used the following buffers (each at 20 mM, Sigma-Aldrich): for pH 3.0, no buffer; for pH 4.0 and 5.0, HOMOPIPES buffer; for pH 5.5–6.5, MES buffer; for pH 7.0, PIPES buffer; for pH 7.5–8.0, HEPES buffer; for pH 8.5, TAPS buffer; for pH 9.0 and 10.0, CAPSO buffer. Growth of strain Iri35c^T was observed from pH 5.0 to pH 9.0, with an optimum around 7.0–7.5. The pressure range for growth of strain Iri35c^T was tested into high-pressure high-temperature reactors (Top Industrie, Vaux-le-Pénil, France), at 0.1, 10, 20, 30, 40 and 50 MPa, as described previously [30]. The novel isolate was piezophilic, growing from atmospheric pressure (0.1 MPa), to at least 50 MPa, and showed optimal growth at 10–30 MPa. Under optimal growth conditions (80 °C, pH 7.0, 2% NaCl, 5 g l^{-1} S°, and 20 MPa), the doubling time of the novel isolate was 80 min.

Utilization of various individual substrates for growth was tested in a basal medium supplemented with 0.05% (w/v) yeast extract (YE) as growth factor, and without this growth factor, as described previously [11]. The following substrates were tested, at the final concentrations shown in brackets: tryptone (0.5% w/v), peptone (0.5% w/v), yeast extract (0.5% w/v), beef extract (0.5% w/v), casamino acids (0.4% w/v), casein

(0.5% w/v), formate (20 mM), acetate (20 mM), pyruvate (20 mM), fumarate (20 mM), propionate (20 mM), succinate (20 mM), maltose (20 mM), fructose (20 mM), lactose (20 mM), ribose (20 mM), galactose (20 mM) and glucose (20 mM). Positive controls were performed for all tests, and unsupplemented media were used as negative controls. To examine the ability of the strain to grow in the absence of elemental sulphur, cells were cultivated in modified Ravot medium without sulphur. Alternative electron acceptors were also tested in a sulphur-depleted medium, under a gas phase of N_2 (100%, 150 kPa): L-cystine (5 g l^{-1}), polysulphides (0.5 mM), thiosulphate (20 mM), sulphate (20 mM), sulphite (5 mM), nitrate (20 mM), nitrite (5 mM) and dioxygen (0.5, 5, 20% v/v). Growth was monitored over 3 days of incubation. The results were considered positive when growth was still observed after two successive subcultures (1/100th subculturing), on the test medium. All tests were performed in duplicates and growth was confirmed after microscopic observation. Hydrogen sulphide production was monitored with a colorimetric test as described previously [31]. Gas concentrations in the headspace phase were determined using a modified INFICON/MicroGC FUSION Gas Analyser (INFICON, Basel, Switzerland) fitted with a pressure gauge (CTE8005AY0, Sensortechnics GmbH) and two conductivity detectors. Separation was performed using two columns: molecular sieve 10 m column and argon as a carrier gas; and a RT-Q12 m using helium as a carrier gas. Cations and anions produced from peptone and yeast extract fermentation were identified by ionic chromatography on a Dionex ICS-900 Ion Chromatography System (Dionex, Camberley UK) coupled with a CERS 500 4 mm suppressor and a DS5 conductivity detector (40 °C) and fitted with a RFC-10 Reagent-Free Controller, an ASDV autosampler, and an IonPac CS16 column maintained at 60 °C in a UltiMate 3000 Thermostated Column Compartment (Thermo Scientific, Waltham, MA, USA).

In the presence of elemental sulphur and under strict anaerobic conditions, complex carbon sources such as yeast extract, peptone, tryptone and beef extract supported fast and significant growth. Cations and anions produced by the fermentation of peptone and yeast extract included formate, acetate, propionate, isobutyrate, succinate or malate, isovalerate, ammonia, carbon dioxide and hydrogen sulphide. The presence of all or part of these organic acids has already been reported as products of amino acid catabolism in other *Thermococcus* species (*T. gorgonarius*, *T. alcaliphilus*, *T. piezophilus*…) [11, 13, 32]. The production of ammonium, already reported in *T. alcaliphilus* for example, probably results from the transamination or oxidation of amino acids [13]. In the absence of elemental sulphur, the strain produced hydrogen by fermentation of peptone and yeast extract. Under our experimental conditions, no obvious growth was observed with the other carbon sources tested, with the exception of maltose which slightly enhanced growth. Poor growth was observed on peptone and yeast extract under pure fermentation conditions, in the absence of sulphur species. Although not necessary for growth, L-cystine and elemental sulphur clearly stimulated the growth of the strain, and were both reduced to hydrogen sulphide. None of the other sulphur species tested (sulphate, thiosulphate, sulphite, polysulphides) had an effect on growth. Nitrate, nitrite and oxygen (aerobic to microaerophilic conditions) were not used by the cells as terminal electron acceptors. Growth by carboxydotrophy was not tested as the gene encoding the carbon monoxide dehydrogenase CooF, one central protein in the *Thermococcales*'s carbon monoxide metabolism, was absent from the genome. Similarly, growth on chitin and starch has not been tested because the degradation pathways of these compounds are incomplete based on the MetaCyc database.

The annotation of the Iri35c^T genome confirmed that the strain has the genetic potential to grow organoheterotrophically from peptides, amino acids and carbohydrates. Concerning the catabolism of peptides and amino acids, the genome encodes several proteases and two central enzymes involved in the oxidation of amino acids into their respective organic acids: the alanine aminotransferase (AlaAT; TIRI35C_0605) and the glutamate dehydrogenase (GDH; TIRI35C_0530). It has been proposed that these two enzymes, AlaAT and GDH, may act in a coordinated manner to maintain the redox balance in *Thermococcales* metabolism and to form an electron sink, by modulating the transformation of pyruvate towards acetate or alanine; alanine could be accumulated as an end-product under high H_2 partial pressure and in the absence of sulphur [33, 34]. Based on MicroCyc, the degradation pathways for six amino acids are complete in the genome of strain Iri 35c^T: alanine, arginine, asparagine, aspartate, glutamine and glycine. These results are congruent with the growths observed on complex proteinaceous substrates. With regard to the catabolism of sugars, the genome contains notably an ABC-type maltose/maltodextrin transport system MalEFGK (TIRI35C_0120- TIRI35C_0122–0124), a cyclomaltodextrinase (TIRI35C_0125), three alpha-amylases (TIRI35C_1801; TIRI35C_1925; TIRI35C_2170), one pullullanase (TIRI35C_0121) and a complete Embden-Meyerhof-Parnas pathway V (including three enzymes unique to this modified version of the glycolysis pathway, typically found within *Thermococcales* [35], namely: an ADP-dependent glucokinase (locus tag TIRI35C_0588), an ADP-dependent phosphofructokinase (TIRI35C_1487) and a glyceraldehyde-3-phosphate:ferredoxin oxidoreductase (TIRI35C_2006)). It encodes also full degradation pathways for glycerol, D-mannose and melibiose. In *Thermococcales* the catabolism of carbohydrates produces reducing equivalents as reduced ferredoxins, while the catabolism of amino acids produces both NADPH and reduced ferredoxins. Reduced ferredoxins are used by two main types of membrane-bound hydrogenases, Mbh and Mbs (previously termed Mbx), which conserve energy by creating an ion gradient across the membrane, and this gradient can then be used by an ATP synthase (TIRI35C_0071–0079) to produce energy [36]. These two main types of membrane-bound hydrogenases, which are respectively hydrogenogenic (Mbh) and sulphidogenic (Mbs) are mobilized according to whether or not there is sulphur in the culture medium [36]. Both are present in the

genome of strain Iri35c^T (Mbh: TIRI35C_2078–2097; Mbs: TIRI35C_0918–0921, TIRI35C_0406–0409). In addition to these membrane-bound hydrogenases, the genome encodes also cytosolic hydrogenases. The gene cluster encoding the formate hydrogenlyase complex which is present in several *Thermococcales*, combines H_2 oxidation with CO_2 reduction to form formate (which could mitigate H_2 saturation under hydrogenogenic growth conditions) [37], is incomplete in this draft genome, which suggests that formate is produced by another pathway in this strain. However, the genome codes for the pyruvate formate lyase activating-enzyme (TIRI35C_0782) which allows the conversion of pyruvate (the end-product of glycolysis) into formate and acetyl Co-A, and might explain the formate production as a catabolic end-product.

In summary, like many *Thermococcales*, strain Iri35c^T is a strict anaerobic archaeon growing chemoorganoheterotrophically on complex proteinaceous substrates, whose growth is very largely stimulated by the presence of elemental sulphur or L-cystine to detoxify the dihydrogen produced by its metabolism (deleterious to its growth) and thus produce a high biomass in culture.

As is very often observed in the genus *Thermococcus*, which forms a fairly homogeneous group in terms of physiology, there are very few phenotypic differences between strain Iri35c^T and its phylogenetically closely related species. These differences are summarized in Table 1. Strain Iri35c^T is distinguishable from *T. pacificus* by the fact that it does not require the presence of sulphur to grow. In addition, the novel isolate has a slightly lower optimal growth temperature than the majority of its closest relatives.

Therefore, from the clear genotypic distance, the many physiological similarities and some phenotypical differences, we comply with the phylo-phenetic concept that currently prevails for the description of a new species. Thus, we proposed to assign strain Iri35c^T to a novel species, for which the name *Thermococcus camini* sp. nov. is proposed.

DESCRIPTION OF *THERMOCOCCUS CAMINI* SP. NOV.

Thermococcus camini (ca.mi'ni. L. gen. n. *camini* of a furnace, referring to the isolation of the type strain from a hydrothermal chimney).

Cells are irregular motile cocci (diameter: 1.1±0.2 µm). Obligately anaerobic. Growth is observed at temperatures between 50 and 90 °C (optimum 75–80 °C), at NaCl concentration from 1–5% (optimum 2%) and at pH from 5.0 to 9.0 (optimum 7.0–7.5). Piezophilic growing optimally under 10–30 MPa. S° or L-cystine is not required for growth but definitely stimulates growth. Does not use sulphate, thiosulphate, sulphite, polysulphide, nitrate, nitrite or oxygen (0.5, 5, 20% v/v) as electron acceptors. Chemoorganoheterotrophic growth occurs on complex proteinaceous substrates (yeast extract, peptone, tryptone, beef extract) and growth is slightly enhanced by maltose addition. Fermentation products (on peptone, yeast extract and S°) include isovalerate, isobutyrate, acetate, propionate, formate, succinate/or malate, ammonia, thiosulphate, carbon dioxide and hydrogen sulphide.

The type strain Iri35c^T (=UBOCC M-2026^T=DSM 111003^T) was isolated from a deep-sea chimney rock sample collected at a depth of 2300 m from a hydrothermal chimney at the Rainbow vent field, Mid-Atlantic Ridge. The genomic DNA G+C content of the type strain is 54.63 mol%.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and draft genome sequences are MT921160 and LR881183.1, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Godfroy A, François D, Hartunians J, Moalic Y, Alain K. (in press). Vetriani C and Giovannelli D (eds). In: *The Microbiology of Deep-Sea. Physiology, Metabolism and Ecology of Thermophiles from Deep-sea Vents*. Springer International;
2. Bertoldo C, Antranikian G. The order *Thermococcales*. Dworkin M, Falkow S, Rosenberg E, Schleifer KH and Stackerbrandt E (eds). In: *The Prokaryotes*, Vol. 3. New York: Springer; 2006. pp. 69–81.
3. Kozhevnikova DA, Taranov EA, Lebedinsky AV, Bonch-Osmolovskaya EA, Sokolova TG. Hydrogenogenic and sulfidogenic growth of *Thermococcus* archaea on carbon monoxide and formate. *Microbiology* 2016;85:400–410.
4. Sokolova TG, Jeanthon C, Kostrikina NA, Chernyh NA, Lebedinsky A, *et al.* The first evidence of anaerobic CO oxidation coupled with H2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Extremophiles* 2004;8:317–323.
5. Atomi H, Reeve J. Microbe Profile: *Thermococcus kodakarensis*: the model hyperthermophilic archaeon. *Microbiol* 2019;165:1166–1168.
6. Thiel A, Michoud G, Moalic Y, Flament D, Jebbar M. Genetic manipulations of the hyperthermophilic piezophilic archaeon *Thermococcus barophilus*. *Appl Environ Microbiol* 2014;80:2299–2306.

7. Atomi H, Fukui T, Kanai T, Morikawa M, Imanaka T. Description of *Thermococcus kodakaraensis* sp. nov., a well studied hyperthermophilic archaeon previously reported as *Pyrococcus* sp. *Archaea* 2004;1:263–267.
8. González JM, Kato C, Horikoshi K. *Thermococcus peptonophilus* sp. nov., a fast-growing, extremely thermophilic archaebacterium isolated from deep-sea hydrothermal vents. *Arch Microbiol* 1995;164:159–164.
9. Zhao W, Zeng X, Xiao X. *Thermococcus eurythermalis* sp. nov., a conditional piezophilic, hyperthermophilic archaeon with a wide temperature range for growth, isolated from an oil-immersed chimney in the Guaymas Basin. *Int J Syst Evol Microbiol* 2015;65:30–35.
10. Jolivet E, L'Haridon S, Corre E, Forterre P, Prieur D. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *Int J Syst Evol Microbiol* 2003;53:847–851.
11. Dalmasso C, Oger P, Selva G, Courtine D, L'Haridon S, *et al*. *Thermococcus piezophilus* sp. nov., a novel hyperthermophilic and piezophilic archaeon with a broad pressure range for growth, isolated from a deepest hydrothermal vent at the Mid-Cayman Rise. *Syst Appl Microbiol* 2016;39:440–444.
12. Marteinsson VT, Birrien JL, Reysenbach AL, Vernet M, Marie D, *et al*. *Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* 1999;49:351–359.
13. Keller M, Braun FJ, Dirmeier R, Burggraf S, Rachel R, *et al*. *Thermococcus alcaliphilus* sp. nov., a new hyperthermophilic archaeum growing on polysulfide at alkaline pH. *Arch Microbiol* 1995;164:390–395.
14. Zeng X, Birrien JL, Fouquet Y, Cherkashov G, Jebbar M, *et al*. Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure–temperature limits for life. *ISME J* 2009;3:873–876.
15. Eren AM, Vineis JH, Morrison HG, Sogin ML. A filtering method to generate high quality short reads using illumina paired-end technology. *PLOS ONE* 2013;8:e66643.
16. Minoche AE, Dohm JC, Himmelbauer H. Evaluation of genomic high-throughput sequencing data generated on Illumina HiSeq and Genome Analyzer systems. *Genome Biol* 2011;12:R112.
17. Vallenet D, Calteau A, Dubois M, Amours P, Bazin A, *et al*. MicroScope: an integrated platform for the annotation and exploration of microbial gene functions through genomic, pangenomic and metabolic comparative analysis. *Nucleic Acids Res* 2020;48:D589.
18. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, *et al*. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
19. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7 improvements in performance and usability. *Mol Biol Evol* 2013;30:772–780.
20. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evol Biol* 2010;10:210.
21. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, *et al*. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 2010;59:307–321.
22. Lefort V, Longueville J-E, Gascuel O. SMS: Smart Model Selection in PhyML. *Mol Biol Evol* 2017;34:2422–2424.
23. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019;47:W259.
24. Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
25. Richter M, Rosselló-Móra R, Glöckner FO, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
26. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
27. Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
28. Wayne LG, Stackebrandt E, Kandler O, Colwell RR, Krichevsky M, *et al*. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 1987;37:463–464.
29. Rosselló-Móra R, Whitman WB. Dialogue on the nomenclature and classification of prokaryotes. *Syst Appl Microbiol* 2019;42:5–14.
30. Alain K, Marteinsson VT, Miroshnichenko M, Bonch-Osmolovskaya E, Prieur D, *et al*. *Marinitoga piezophila*, sp. nov., a rod-shaped thermopiezophilic bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* 2002;52:1331–1339.
31. Cord-Ruwisch R. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *Journal of Microbiological Methods* 1985;4:33–36.
32. Miroshnichenko ML, Gongadze GM, Rainey FA, Kostyukova AS, Lysenko AM, *et al*. *Thermococcus gorgonarius* sp. nov. and *Thermococcus pacificus* sp. nov.: heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. *Int J Syst Bacteriol* 1998;48:23–29.
33. Kengen SWM, Stams AJM. Formation of l-alanine as a reduced end product in carbohydrate fermentation by the hyperthermophilic archaeon *Pyrococcus furiosus*. *Arch Microbiol* 1994;161:168–175.
34. Ward DE, Kengen SW, van Der Oost J, de Vos WM. Purification and characterization of the alanine aminotransferase from the hyperthermophilic archaeon *Pyrococcus furiosus* and its role in alanine production. *J Bacteriol* 2000;182:2559–2566.
35. Sakuraba H, Goda S, Ohshima T. Unique sugar metabolism and novel enzymes of hyperthermophilic archaea. *Chem Record* 2004;3:281–287.
36. Lipscomb GL, Schut GJ, Scott RA, Adams MWW. SurR is a master regulator of the primary electron flow pathways in the order Thermococcales. *Mol Microbiol* 2017;104:869–881.
37. Topçuoğlu BD, Meydan C, Orellana R, Holden JF. Formate hydrogenlyase and formate secretion ameliorate H2 inhibition in the hyperthermophilic archaeon *Thermococcus paralvinellae*. *Environ Microbiol* 2018;20:949–957.
38. Kuwabara T, Minaba M, Ogi N, Kamekura M. *Thermococcus celericrescens* sp. nov., a fast-growing and cell-fusing hyperthermophilic archaeon from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* 2007;57:437–443.
39. Grote R, Li L, Tamaoka J, Kato C, Horikoshi K, *et al*. *Thermococcus siculi* sp. nov., a novel hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Okinawa Trough. *Extremophiles* 1999;3:55–62.
40. Hensley SA, Jung JH, Park CS, Holden JF. *Thermococcus paralvinellae* sp. nov. and *Thermococcus cleftensis* sp. nov. of hyperthermophilic heterotrophs from deep-sea hydrothermal vents. *Int J Syst Evol Microbiol* 2014;64:3655–3659.
41. Holden JF, Takai K, Summit M, Bolton S, Zyskowski J, *et al*. Diversity among three novel groups of hyperthermophilic deep-sea *Thermococcus* species from three sites in the northeastern Pacific Ocean. *FEMS Microbiol Ecol* 2001;36:51–60.